

Nonfruiting Host Tree Volatile Blends: Novel Attractants for the Plum Curculio (Coleoptera: Curculionidae)

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ABSTRACT We evaluated the attractiveness of novel synthetic host plant volatiles alone and in combination with the synthetic aggregation pheromone, grandisoic acid (GA), to overwintered adult plum curculio, *Conotrachelus nenuphar* (Herbst) (Coleoptera: Curculionidae) in association with black masonite pyramid traps deployed in an unsprayed apple orchard in 2003 and 2004. Synthetic host plant volatiles evaluated included (1) those identified from foliar and woody tissues of a nonfruiting Stanley plum tree (6-Tree) or the two major components (2-Tree); (2) those identified from Stanley plum fruit (7-Plum) or two components eliciting positive GC-EAD response from plum curculios (2-Plum); and (3) benzaldehyde formulated into rubber septa and replaced weekly [Ben(s)] or dispensed into polyethylene vials and replaced weekly [Ben(r)], not replaced [Ben(nr)], and formulated with 10% 1,2,4-trichlorobenzene and not replaced [Ben(TCB)]. In 2003, the greatest numbers of plum curculios were captured in traps baited with 6-Tree + GA and Ben(TCB) + GA, with responses to these baits significantly greater than responses to unbaited control traps. In 2004, the greatest number of plum curculios was captured in traps baited with 6-Tree(h) (high release rate dispenser) + Ben(TCB) + GA, with response to this bait significantly greater than responses to unbaited traps. Combined results indicate that volatiles released by foliar and woody tissues of plum are at least as attractive as the single fruit-based attractant, benzaldehyde, when combined with GA and point toward more competitive attractants for plum curculio based on multiple component blends of volatiles released by the entire tree.

KEY WORDS *Conotrachelus nenuphar*, plum curculio, weevil, attractants, pheromone

BEHAVIORALLY BASED MONITORING AND management strategies include attracticides, the deployment of a long-distance attractant with a killing agent, and/or collection device (Foster and Harris 1997). Host plant volatiles serve as attractants for many phytophagous insects (Bernays and Chapman 1994); these olfactory stimuli aid in location of food, mates, and/or oviposition sites (Visser 1986). Pheromones also serve as attractants for conspecifics of the same and/or opposite sex (Cardé and Baker 1984). For some species, responses to these attractants can be enhanced because of synergy between host plant volatiles and male-produced aggregation pheromones, notably in the family Curculionidae (Landolt 1997, Landolt and Phillips 1997). For example, such responses have been recorded for the palmetto weevil, *Rhyncophorus*

cruentatus (F.) (Weissling et al. 1994) and the West Indian sugarcane weevil, *Metamasius hemipterus* (Ramirez-Lucas et al. 1996). These synergistic responses have translated into successful monitoring strategies for *R. palmarum* L. (Oehlschlager et al. 1993) and *M. hemipterus* (Giblin-Davis et al. 1996), and more recently, into attracticide-based mass trapping strategies for control of *R. palmarum* L. (Oehlschlager et al. 2002).

The plum curculio, *Conotrachelus nenuphar*, is a serious pest of stone and pome fruit in eastern North America (Racette et al. 1992, Vincent et al. 1999), and there has been a great deal of effort directed toward development of a trap-based monitoring system (Teddies and Wood 1994, Mulder et al. 1997, Prokopy and Wright 1998, Prokopy et al. 2000, Piñero et al. 2001, Leskey and Prokopy 2002) for use against plum curculio in commercial orchards (Johnson et al. 2002, Prokopy et al. 2003, Leskey and Wright 2004a).

Olfactory responses of plum curculios are likely important components of host- and mate-finding because this species moves from overwintering sites at the periphery of orchards into host trees (Smith and Flessel 1968, Lafleur and Hill 1987, Piñero et al. 2001) before fruit set (Piñero et al. 2001) and mates within or beneath host fruit trees (Smith and Salkeld 1964,

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Le Blanc 1982, Racette et al. 1992). Furthermore, adult antennae possess sensilla types consistent with those known to be olfactory receptor neurons in other insects, including the presence of type IV, thought to be for pheromone reception (Alm and Hall 1986). Thus, identification of olfactory cues that may serve as attractants is an important component of research toward development of plum curculio monitoring strategies and trapping systems.

Volatile collections from male plum curculios have yielded a single compound found to be attractive to both males and females, thus considered to be an aggregation pheromone and termed grandisoic acid (GA) (Eller and Bartelt 1996). Furthermore, behavioral studies showed that plum curculios use olfactory cues to locate host fruit trees (Butkewich and Prokopy 1993, Leskey and Prokopy 2001). Additional laboratory studies revealed that host apple and plum odors released by plant tissues between bloom and 2 wk after bloom were attractive to plum curculios (Leskey and Prokopy 2000). Compounds identified from cultivated plum collected 2 wk after bloom (Leskey et al. 2001) and apple collected 1–3 (Prokopy et al. 2001) and 1–4 wk after bloom (Boevé et al. 1996) were tested in the laboratory (Leskey et al. 2001) and in the field (Leskey et al. 2001, Prokopy et al. 2001) for plum curculio attraction. Several compounds, particularly ethyl isovalerate, limonene, benzaldehyde, benzyl alcohol, decanal, *trans*-2-hexenal, geranyl propionate, and hexyl acetate, were found to be attractive (Leskey et al. 2001, Prokopy et al. 2001). When benzaldehyde was evaluated in combination with GA in association with traps under field conditions, plum curculio captures increased significantly compared with captures in traps baited with GA alone or in unbaited traps (Piñero et al. 2001). Only benzaldehyde synergized plum curculio responses to GA in a field experiment evaluating traps baited with benzaldehyde, ethyl isovalerate, and limonene alone or in combination with GA (Piñero and Prokopy 2003). Thus, like other species of curculionids (Landolt 1997, Landolt and Phillips 1997), the combination of synthetic host plant volatiles and aggregation pheromone results in a synergistic response from plum curculios.

Although synergism between benzaldehyde and GA results in increased trap captures, traps baited with these attractants failed to reliably monitor plum curculio activity in northeastern apple and mid-Atlantic apple and peach orchards (Prokopy et al. 2003, Leskey and Wright 2004a). In fact, captures in baited traps declined rapidly after fruit set (Prokopy et al. 2003, Leskey and Wright 2004a), indicating that volatiles released by rapidly developing fruit could be outcompeting synthetic attractants. Indeed, Leskey and Wright (2004b) found that presence of host apple trees did have a significant impact on plum curculio responses to baited traps, further strengthening the hypothesis that olfactory cues produced by host trees, particularly after fruit set, are more attractive to plum curculios than synthetic attractants. Thus, there appears to be a clear need for a more competitive at-

tractant for plum curculio, particularly with regard host plant volatiles.

There are likely a number of weaknesses associated with the approach taken toward identification of host plant-based attractants for plum curculios that could account for failures associated with baited traps. First, the compounds that have been identified thus far include those identified from the fruit only (Leskey et al. 2001, Prokopy et al. 2001). However, plum curculio attraction to odors of foliar and woody apple tissues has been documented (Leskey and Prokopy 2000) and likely exists for these same plum tissues as well. Thus, as plum curculios move from overwintering sites to locate host trees, they encounter and likely respond to host volatiles emanating from the entire tree, not just the fruit, particularly because they arrive in orchards well before fruit is available (Lafleur and Hill 1987, Piñero et al. 2001, Leskey and Wright 2004a). Second, single-component fruit-based attractants may not be very competitive within the context of an orchard, particularly when the attractant itself also is released by developing fruit. For example, although benzaldehyde was identified from plum (Leskey et al. 2001), it also is produced by apple blossoms (Buchbauer et al. 1993). It is likely that volatile release of benzaldehyde and other compounds by host trees contributes to reduction of plum curculio captures in benzaldehyde-baited traps when traps are in close proximity to host trees (Leskey and Wright 2004b). Third, in all identifications of host volatiles to date, fruit were removed from trees and taken to the laboratory for analysis (Boevé et al. 1996, Leskey et al. 2001, Prokopy et al. 2001), a procedure that could alter both the components and rate of volatile release. Finally, the best currently known synthetic host volatile, benzaldehyde (Piñero et al. 2001, Piñero and Prokopy 2003) visibly degrades under field conditions (Piñero et al. 2002), and thus must be replaced weekly (Leskey and Wright 2004a).

Therefore, we conducted experiments to (1) determine if plum curculios are attracted to volatiles released by foliar and woody tissues of plum, as this is a preferred host; (2) compare plum curculio attraction to volatiles identified from on-tree collections of foliar and woody nonfruiting plum tree tissues with those identified from plum fruit removed from fruiting plum trees; and (3) assess degradation of benzaldehyde and explore a means to stabilize this compound for field deployment.

Materials and Methods

Volatile Collection and Identification. Headspace volatiles were collected from foliar and woody tissues of a potted nonfruiting Stanley plum tree in 2002. A large polyethylene bag (48.26 by 58.42 cm, Reynolds Oven Bags; Reynolds, Richmond, VA) was used as a source containment device placed over select branches (including foliage and branches) and sealed with plastic ties. A second volatile collection was taken from 44 green plum fruits removed from Stanley plum trees in 2002. For this collection, plums

Table 1. Synthetic components and ratios comprising experimental lures based on volatile collections taken in situ from foliar and woody tissues of a nonfruiting Stanley plum tree and taken from plum fruit removed from a Stanley plum tree

Compound	6-Tree	2-Tree	7-Plum	2-Plum
β -Caryophyllene	1		8	
4,8-Dimethyl-1,3,7-nonatriene	7	1	3	
Farnesene			10	
Z-3-hexenyl acetate	3			
Z-jasmone ^a			2	1
Limonene	1			
Methyl eugenol ^a			6	3
Methyl salicylate	1		21	
E- β -ocimene	28	4	3	

^a Positive GC-EAD responses from adult plum curculios.

(≈ 20 mm in diameter and 4–5 wk after petal fall) were picked from fruiting Stanley plum trees located within a mixed fruit orchard planted in 1997 at the Appalachian Fruit Research Station (Kearneysville, WV). The orchard received no insecticide and minimal fungicide applications. All plums were removed from trees early in the morning, placed in a cooler, and taken directly to Beltsville, MD, where they were immediately introduced into a 1-liter, four-necked glass container (Zhang et al. 1994), ≈ 2 h after being removed from the trees. Air was drawn into the bag or container through 6–14 mesh activated charcoal (Fisher, Pittsburgh, PA), and out of the bag or container through a trap (15 by 1.5 cm OD) containing Super Q (200 mg each; Alltech Associates, Deerfield, IL) by vacuum (≈ 1 liter/min). Volatiles were aerated continuously for 24 h at room temperature and 16 L: 8 D photoperiod. Volatile collections were eluted with methylene chloride (4 by 0.5 ml); the eluates (2 ml/each sample) were concentrated to ≈ 20 μ l under nitrogen and stored at -30°C for future analysis. A Hewlett-Packard (HP) 6890 gas chromatograph (GC) equipped with a 60 m by 0.25 mm ID, 0.25 μ m film-thickness DB-WAXETR capillary column (J&W Scientific, Folsom, CA) in the splitless mode with hydrogen as carrier (1.4 ml/min) was used for GC analysis (80°C for 2 min, programmed to 250°C at 15°C/min and held for 15 min). The coupled GC-electroantennographic detection (GC-EAD) system used was as previously described (Zhang et al. 1997, 1999), and GC temperature program followed the above protocol. Electronic impact (EI) GC-mass spectrometry (GC-MS) was conducted on a HP 6890 GC coupled to a HP 5973 Mass Selective Detector using an identical DB-WAXETR capillary column (50°C for 2 min, programmed to 230°C at 15°C/min and held for 15 min for regular analysis) or a DB-5 capillary column (60 m by 0.25 mm ID, 0.25 μ m film-thickness; J&W Scientific; 50°C for 2 min, programmed to 300°C at 15°C/min and held for 50 min) but with helium as carrier gas. A 70-eV electron beam was employed for sample ionization. Nine compounds were identified from foliar and woody tissue and plum fruit samples (Table 1); results were confirmed by comparison of mass spectra and GC retention times with synthetic standards.

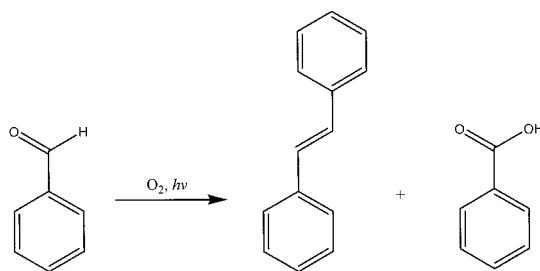


Fig. 1. Degradation of benzaldehyde.

Benzaldehyde Analysis. When neat benzaldehyde is exposed to the outdoor environment in a closed low-density polyethylene vial as formulated by Leskey and Wright (2004a), it will eventually solidify into crystals of *trans*-stilbene (mp 124°C) and benzoic acid (mp 122°C) as determined by ^1H -NMR analysis and melting point (M.H., unpublished data) (Fig. 1). The crystals have a yellowish color because of trace amounts of *cis*-stilbene (mp -5°C). By exposing neat benzaldehyde to light and air at a constant temperature of 50°C (122°F) in a glass vial, it is possible to accelerate the degradation and obtain an ≈ 90 –95% solid mass in 48 h with the same degradative products, *trans*-stilbene and benzoic acid. Using this accelerated method, a series of additives was examined for effectiveness in preventing the build-up of solids in the benzaldehyde with the working assumption that the solid formation was the cause of decreased benzaldehyde emissions. Additives that were evaluated included high surface area particles (activated carbon and molecular sieves), high vapor pressure solvents (acetone, hexane, and toluene), low vapor pressure, viscous liquids (mineral and silicone oils), and a low vapor pressure, low viscosity solvent 1,2,4-trichlorobenzene (TCB). Only TCB appeared to be effective. During a representative study, a 9:1 benzaldehyde:TCB (wt:wt) solution was loaded into a low-density polyethylene vial and capped. The vial was stored at 50°C at a location near a window to allow sunlight exposure on the vial. A control vial of neat benzaldehyde was placed next to the test vial. The weights of the vials were taken at intervals to determine the amount of vapor release. After 48 h, the control vial did not exhibit any weight change indicating that the pores of the low-density polyethylene vial were completely clogged. The test vial exhibited a linear ($R^2 = 0.9942$) decrease in weight for a period of 168 h. This suggests that the TCB was successful in solvating the degradation products from benzaldehyde and keeping pores in the low-density polyethylene vial clear. Therefore, TCB was chosen for further study under field deployment conditions as a means to stabilize benzaldehyde but not adversely affect plum curculio attraction to this particular synthetic bait.

Synthetic Baits

Plum-based Treatments. In 2003, synthetic volatile blends based on natural ratios of six components iden-

tified from intact, nonfruiting, on-tree volatile collections of foliar and woody tissues (6-Tree), seven components identified from plum fruit-only samples removed from a fruiting plum tree (7-Plum), as well as the two major components identified from the nonfruiting on-tree sample (2-Tree) and two components identified from the plum fruit-only sample that elicited a positive GC-EAD response from plum curculios (2-Plum; Table 1) were formulated as neat chemical blends into centrifuge tubes with 80 μ l of total material. The cap of the centrifuge tubes were opened for field deployment and replaced weekly. These were tested alone and in combination with GA obtained from ChemTica International (San Jose, Costa Rica). Release rate of GA dispensers (25 mg) was determined by the manufacturer to be \approx 1 mg/d.

In 2004, 500 μ l of each neat chemical blend of 6-Tree, 7-Plum, and 2-Plum were dispensed into 1-ml white, UV-resistant low-density polyethylene vials (Wheaton Scientific Products, Millville, NJ) to create a higher release rate than that of 2003 dispensers; these are referred to as 6-Tree(h), 7-Plum(h), and 2-Plum(h). Vials of 6-Tree(h) were capped with a drop dispenser (Wheaton Scientific Products) with a 4 cm length of 0.4-mm-wide cotton wick, with \approx 2 cm exposed, creating a 18.4-mg/d release rate (determined gravimetrically at 25°C). Vials of 7-Plum(h) and 2-Plum(h) were capped with drop dispenser lids (Wheaton Scientific Products). A hole was drilled through the top of each lid, and a 4 cm length of 1.6-mm-wide wick was pulled through the hole. For 7-Plum(h) and 2-Plum(h), 0.5- and 1-cm pieces of wick were left exposed, respectively. This procedure resulted in release rates of 7-Plum(h) at 63.2 mg/d and 2-Plum(h) at 20.9 mg/d, again determined gravimetrically at 25°C. The treatments 6-Tree(h), 7-Plum(h), and 2-Plum(h) were evaluated alone and in combination with GA. Also, 6-Tree, 7-Plum, and 2-Plum were formulated into gray rubber septa to provide for a low release rate comparison. Gray halobutyl rubber septa (5 mm) were loaded with 10 mg of candidate blend, \approx 40 μ l of hexane, and two drops of butylated hydroxytoluene (BHT) solution (10 mg/ml hexane). After loading, the solvent was allowed to evaporate in a fume hood for 30 min. Lures were wrapped in aluminum foil, stored in 20-ml plastic vials, and shipped by express carrier on the same day. On arrival, the lures were kept in a freezer at -30°C until use. They are referred to as 6-Tree(l), 7-Plum(l), and 2-Plum(l), with release rates of 7.0, 7.7, and 6.0 mg/d, respectively, determined gravimetrically at 25°C. Each was evaluated in combination with GA only and replaced weekly (based on the likelihood of lower release rates because of lower temperatures in nature). GA dispensers were obtained from ChemTica International; release rate of GA dispensers (35 mg) was determined by the manufacturer to be \approx 1 mg/d.

Benzaldehyde-based Treatments. In 2003, benzaldehyde alone (Ben) or in combination with 10% TCB [Ben(TCB)] was formulated into 1-ml white, UV-resistant low-density polyethylene vials (Wheaton Scientific Products). For benzaldehyde formulated in

UV-resistant vials without TCB, two treatments were evaluated: (1) weekly replacement of dispensers [Ben(r)], and (2) no replacement throughout the entire study [Ben(nr)]. Benzaldehyde alone also was formulated into rubber septa [Ben(s)]. All benzaldehyde dispensers were deployed alone and in combination with GA, with the exception of dispensers of Ben(TCB) and Ben(nr), which were not replaced throughout the entire experiment and were deployed in combination with GA only.

In 2004, the same treatments were evaluated with the exception of Ben(s), which was not included in the study because of poor results in 2003. An additional treatment was added in 2004, Ben(TCB) in combination with 6-Tree(h) and GA.

Bait Deployment. All baits were deployed in association with standard black masonite pyramid traps deployed within an unmanaged apple orchard (Fig. 2) to maximize the effect of competition from natural sources of olfactory stimuli, i.e., apple trees (Leskey and Wright 2004b). Baits were attached to the outer surface of the collection device near the top of the pyramid traps. The pyramid traps were positioned between large standard 'Stayman' and 'Red Delicious' apple trees at spacing of 8.5 by 6.8 m. Four replicates of each bait were deployed. Within each replicate, bait location was randomly assigned. In 2003, baited traps were deployed on 4 April at half inch green and checked weekly through 15 May when developing apple fruit were \approx 14 mm in diameter. Bait locations were rerandomized within each replicate every 7–14 d. In 2004, baited traps were deployed on 16 April at pink and checked weekly through 20 May when developing fruit were \approx 20 mm in diameter. Bait locations were rerandomized within each replicate every 7 d.

Statistical Analysis. Nontransformed data, because the homogeneity-of-variances assumption was met in all cases, were analyzed using the general linear model (GLM) procedure (SAS Institute, Cary, NC) to construct analysis of variance (ANOVA) tables for cumulative trap captures recorded over the entire trapping period. The model included the following class variables: bait combination and replicate. When the GLM indicated significant differences, multiple comparisons were calculated using Fisher's least significant difference (LSD; $P < 0.05$). To measure attractiveness of a particular compound, a response index (Leskey et al. 2001) was calculated by subtracting the number of plum curculios responding to the control (C; unbaited traps) from the number responding to the treatment (T; traps baited with a particular compound) dividing by the total number of plum curculios captured in the treatment and control traps, and multiplying by 100. Thus, $RI = [(T - C) / (T + C)] \times 100$; the greater the RI, the more attractive the compound. The RI for each compound was calculated separately for each replicate and presented as a mean RI value. To analyze the extent of response to traps baited with a particular compound compared with unbaited control traps, data were subjected to a one-tailed Wilcoxon rank sum test ($P < 0.05$). For each particular

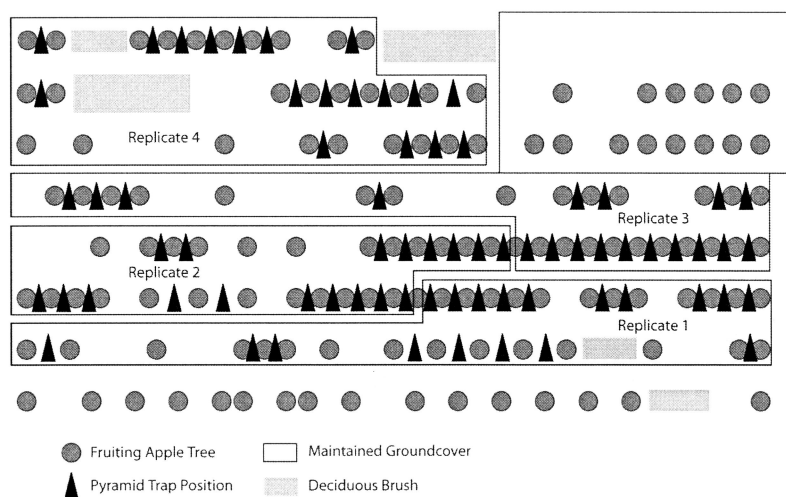


Fig. 2. Unsprayed apple orchard in which field trials were conducted (Summit Point, WV).

compound, the total number of plum curculios that entered baited traps and unbaited traps were ranked among all replicates, and ranks were totaled for the treatment and the control and ultimately used to calculate the test statistic (Statistix 2003).

Results

2003. We captured a total of 711 adult plum curculios during a 42-d trapping period from 4 April to 15 May. In the early season, from half inch green through bloom (4–24 April), we captured 166 adult plum curculios. We captured 311 adults during petal fall (25 April to 1 May) and 234 adults from fruit set until developing apple reached 14 mm in diameter (2–15 May). The GLM for overall plum curculio captures was significant ($F = 3.18$; $df = 18,45$; $P < 0.01$). There was a significant effect of bait treatment ($P = 0.05$). Replicate also was significant ($P < 0.01$), with more plum curculios located in replicates 2 and 4, located in regions of the orchard with hedgerows and ideal overwintering sites (Fig. 2). The greatest number of plum curculios were captured in traps baited with 6-Tree + GA (RI = 35.7); trap captures with this treatment along with Ben(TCB) + GA (RI = 35.1) were significantly greater than traps baited with 7-Plum, 2-Tree, 7-Tree, or 2-Plum alone, as well as GA alone, Ben(s) + GA, 2-Tree + GA, and unbaited traps (Table 2). Intermediate levels of attraction were recorded for 7-Plum + GA (RI = 25.8), Ben(r) + GA (RI = 25.0), and 2-Plum + GA (RI = 23.4). Mean captures in traps baited with 6-Tree + GA and Ben(TCB) + GA were generally much higher than that for unbaited traps throughout the trapping period, with captures extending well beyond petal fall (Fig. 3).

2004. We captured a total of 1,471 adult plum curculios during a 38-d trapping period from 12 April to 20 May. In the early season, from pink through bloom

(16–22 April), we captured a total of 573 adult plum curculios. During petal fall (23–29 April), we captured 239 adults, and from fruit set until developing apple fruit reached ≈ 20 mm diameter (30 April to 20 May), we captured 659 adults. The GLM for overall plum curculio captures was significant ($F = 6.75$; $df = 17,42$; $P < 0.0001$). As in 2003, the effects of bait treatment were significant ($P = 0.003$), and the effect of replicate was significant ($P < 0.0001$), with significantly more

Table 2. Field response of adult plum curculios to traps baited with different synthetic odor treatments, including mean \pm SE, with plum curculios captured per trap and corresponding response indices for each compound in 2003

Bait	Replicates	Total no. captured	Mean \pm SE ^a	RI
6-Tree + GA	4	76	19.0 \pm 4.7a	35.7 ^b
Ben(TCB) ^c + GA	4	75	18.8 \pm 4.3a	35.1 ^b
7-Plum + GA	4	61	15.5 \pm 7.5ab	25.8
Ben(r) ^d + GA	4	60	15.0 \pm 6.5abc	25.0
2-Plum + GA	4	58	14.5 \pm 3.9abc	23.4
Ben(s) ^e	4	56	14.0 \pm 6.0abcd	21.7
Ben(nr) ^f + GA	4	48	12.0 \pm 3.0abcd	14.3
Ben(r)	4	45	11.3 \pm 3.5abcd	11.1
Unbaited	4	36	9.0 \pm 2.6bcd	0.0
7-Plum	4	35	8.8 \pm 4.5bcd	-1.4
2-Tree	4	33	8.3 \pm 4.1bcd	-4.3
GA	4	31	7.8 \pm 1.9bcd	-7.5
Ben(s) + GA	4	30	7.5 \pm 2.9bcd	-9.1
6-Tree	4	25	6.3 \pm 2.3bcd	-18.0
2-Tree + GA	4	24	6.0 \pm 2.0cd	-20.0
2-Plum	4	18	4.8 \pm 1.3d	-33.3

^a Means in the same column followed by a different letter are significantly different according to Fisher's LSD ($P < 0.05$).

^b Treatments and controls are significantly different from each other at $P < 0.05$ according to a Wilcoxon rank-sum test.

^c TCB was added to vial containing benzaldehyde.

^d Benzaldehyde was replaced weekly throughout the course of the study.

^e Benzaldehyde is formulated into a rubber septum.

^f Benzaldehyde was not replaced throughout the course of the study.

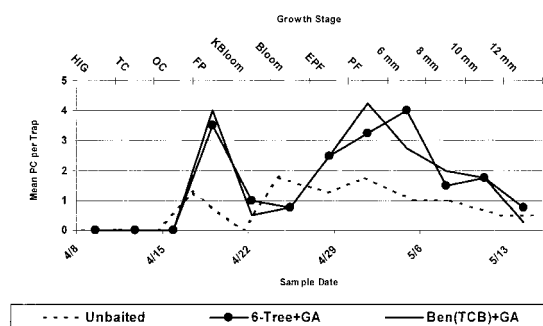


Fig. 3. Mean plum curculio captures per trap from 8 April to 15 May 2003 in unbaited traps and traps baited with 6-Tree + GA, and Ben (TCB) + GA. Abbreviated labels on growth stage reference line, HIG, TC, OC, FP, KBloom, EFP, and PF, refer to half inch green, tight cluster, open cluster, full pink, king bloom, early petal fall, and petal fall, respectively, whereas numeral labels represent apple fruit diameter in millimeters.

adults captured in replicate 4 (Fig. 2). The greatest number of plum curculios were recovered from traps baited with 6-Tree(h) + Ben(TCB) + GA (RI = 49.9), with 173 adults captured (Table 3). Traps baited with this treatment along with Ben(r) + GA (RI = 44.1), Ben(nr) + GA (RI = 34.4), Ben(TCB) + GA (RI = 35.8), and GA alone (RI = 31.9) captured significantly more plum curculios than unbaited traps (Table 3). Traps baited with 6-Tree(h) + GA (115 adults) also captured significantly more adults than

Table 3. Field response of adult plum curculios to traps baited with different synthetic odor treatments, including mean \pm SE, with plum curculios captured per trap and corresponding response indices for each compound in 2004

Bait	Replicates	Total no. captured	Mean \pm SE ^a	RI
6-Tree(h) + Ben (TCB ^f) + GA	4	173	43.3 \pm 11.4a	49.9 ^h
Ben (r ^d) + GA	4	136	34.0 \pm 4.5ab	44.1 ^h
Ben (nr ^e) + GA	4	123	30.8 \pm 8.3abc	34.4
Ben(TCB) + GA	4	119	29.8 \pm 5.6abc	35.8
GA	4	118	29.5 \pm 7.8abc	31.9
6-Tree (h ^f) + GA	4	115	28.8 \pm 15.4bcd	5.0
6-Tree (l ^e) + GA	4	111	27.8 \pm 6.8bcde	30.5
7-Plum(l) + GA	4	91	22.8 \pm 6.6bcdef	23.2
2-Plum	4	86	21.5 \pm 7.6bcdef	18.0
2-Plum(h) + GA	4	79	19.8 \pm 4.4cdef	18.3
7-Plum(h) + GA	4	79	19.8 \pm 7.5cdef	12.8
7-Plum	4	70	17.5 \pm 6.2cdef	5.4
6-Tree	4	60	15.0 \pm 6.7def	-3.7
2-Plum(l) + GA	4	56	14.0 \pm 4.1ef	-2.6
Unbaited	4	55	13.8 \pm 3.3f	0.0

^a Means in the same column followed by a different letter are significantly different according to Fisher's LSD ($P < 0.05$).

^b Treatments and controls are significantly different from each other at $P < 0.05$ according to a Wilcoxon rank-sum test.

^c TCB was added to vial containing benzaldehyde.

^d Benzaldehyde was replaced weekly throughout the course of the study.

^e Benzaldehyde was not replaced throughout the course of the study.

^f High release rate of synthetic compounds.

^g Low release rate of synthetic compounds.

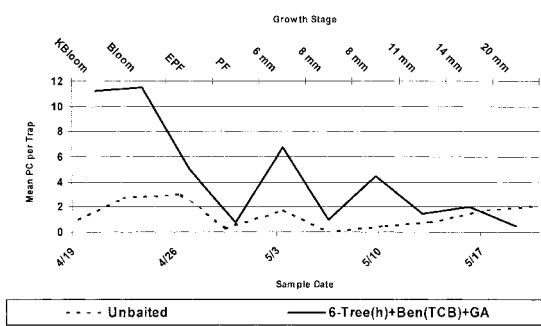


Fig. 4. Mean plum curculio captures per trap from 19 April to 20 May 2004 in unbaited traps and traps baited with 6-Tree(h) + Ben(TCB) + GA. Abbreviated labels on growth stage reference line, HIG, TC, OC, FP, KBloom, EFP, and PF, refer to half inch green, tight cluster, open cluster, full pink, king bloom, early petal fall, and petal fall, respectively, whereas numeral labels represent apple fruit diameter in millimeters.

unbaited traps, although the RI was very low (RI = 5.0) because of very low captures in replicate 3. Mean captures in traps baited with the most attractive bait, 6-Tree(h) + Ben(TCB) + GA, were generally much higher than that for unbaited traps throughout the trapping period, with captures extending well beyond petal fall (Fig. 4).

Discussion

Plum curculio, a fruit-feeding insect, is not only attracted to natural fruit odor (Leskey and Prokopy 2000) and to single-component synthetic fruit volatiles (Leskey et al. 2001, Prokopy et al. 2001), but also to synthetic volatile blends identified from foliar and woody tissues. Studies reported here are the first to show plum curculio attraction to synthetic volatile blends based on headspace collections taken from intact foliar and woody tissues of a nonfruiting plum tree (6-Tree) when in combination with GA (Tables 2 and 3), resulting in the greatest number of plum curculios captured in 2003. Such a strong response to synthetic volatiles identified from foliar and woody tissues in combination with GA indicates that these olfactory cues could be very important in terms of host- and/or mate-finding. Plum curculios generally immigrate from overwintering sites into host fruit trees well before developing fruit is present (Lafleur and Hill 1987, Piñero et al. 2001, Leskey and Wright 2004a), with most females being unmated (Smith and Salkeld 1964, Piñero et al. 2001). Mating occurs in or near host trees (Smith and Salkeld 1964, Le Blanc 1982, Racette et al. 1992). Thus, although plum curculios feed on and oviposit in developing fruit (Quaintance and Jenne 1912, Chapman 1938), they are attracted to volatile cues not only from fruit, but also from the foliar and woody tissues, both of which are more attractive when in association with GA. Similarly, mated female oriental fruit moth, *Cydia molesta* (Busck), which oviposits mainly on peach foliage early in the season (Howitt 1993) and directly on the

fruit later in the season (Hogmire 1995), were attracted to synthetic volatile blends released by peach shoots (Natale et al. 2003).

A common problem encountered in past studies that have attempted to monitor plum curculio activity using traps baited with single fruit volatile, benzaldehyde, in combination with GA is that trap captures decline rapidly after petal fall (Prokopy et al. 2003, Leskey and Wright 2004a). This study is the first to provide evidence for the potential to create more competitive attractants for plum curculio based on multiple component blends of host plant volatiles and/or stable formulations of benzaldehyde. We were able to sustain captures in traps well after petal fall at much higher levels compared with unbaited traps (even under very competitive conditions of within-orchard trap deployment; Leskey and Wright 2004b) with 6-Tree + GA and Ben(TCB) + GA in 2003 (Fig. 2). In 2004, we found that when we combined Ben(TCB) with 6-Tree(h) + GA into a single bait, captures in pyramid traps that included this semiochemical combination resulted greatest numbers of plum curculios captured (173 adults) compared with any other bait treatment (Table 3), with three times as many captures as unbaited traps (only 55 adults). Again, captures were sustained well after petal fall (Fig. 3). In studies on other species of curculionids including the red weevil, *R. ferrugineus* F. (Gunawardena et al. 1998), and *Hylobius pales* (Herbst) (Fettig et al. 1998), adults responded in greater numbers to lures containing multiple rather than single host plant-based components, although they were not tested in combination with a male-produced aggregation pheromone as in this study. Our next step for development of competitive attractants for plum curculio will be to conduct volatile identifications from on-tree headspace collections taken under natural conditions that include fruiting as well as foliar and woody tissues.

We captured greatest numbers of plum curculios in traps baited with 6-Tree + GA and Ben(TCB) + GA followed 7-Plum + GA and Ben(r) + GA in 2003 (Table 2) and 6-Tree(h) + Ben(TCB) + GA and Ben(r) + GA followed by Ben(nr) + GA and Ben(TCB) + GA in 2004 (Table 3). These results confirm previous findings that host plant volatiles in combination with GA are more attractive to plum curculios than if either is deployed alone (Piñero et al. 2001, Piñero and Prokopy 2003, Prokopy et al. 2003, Leskey and Wright 2004a). Piñero et al. (2003) reported a synergistic response to Ben + GA, although we did not observe a synergistic response to Ben(r) + GA in 2003 (identical treatment to that used by Piñero and Prokopy 2003). There are several potential explanations for the differences observed between these studies and those of Piñero et al. (2003). First, Leskey and Wright (2004b) showed that the presence of host apple trees in close proximity to baited traps results in significant reductions in plum curculio captures. Benzaldehyde has been identified from apple blossoms (Buchbauer et al. 1993), and thus, the degree of competition from natural sources of olfactory stimuli, i.e., host apple trees, will likely be greater if baited traps are deployed within an apple orchard (studies reported

here) than if they are deployed outside the orchard (studies by Piñero and Prokopy 2003). A second possibility could be related to the physiological state of plum curculios. Plum curculios captured in studies by Piñero et al. (2003) were likely comprised of individuals that had not previously encountered host trees as they immigrated from externally located overwintering sites into the orchard itself. Conversely, our traps were located within an apple orchard with a nonimmigrating plum curculio population, and therefore, captures in our studies could have been comprised, at least in part, of plum curculio that had encountered host trees before capture. Leskey and Wright (2004b) used plum curculios dislodged from host fruit trees as subjects in a mark-release-recapture experiment and did not observe a synergistic response to traps baited Ben + GA. Finally, variation in responses to Ben + GA may be caused by unknown differences between the univoltine and bivoltine populations of plum curculios inhabiting Massachusetts and West Virginia, respectively (Piñero and Prokopy 2003, Leskey and Wright 2004a).

We know that for plum curculio, volatile release rate and/or concentration may be very important in lure effect. For example, benzaldehyde released at 10 mg/d in combination with GA at 1 mg/d was more attractive than greater or lesser release rates of these two compounds when deployed in association with pyramid traps. However, in association with panel traps, increasing the release rate of GA from 1 to 2 mg/d in combination with benzaldehyde resulted in increased captures (Piñero and Prokopy 2003). In our studies, we deployed GA dispensers that were formulated with 25 mg of total material in 2003 and 35 mg of total material in 2004, but supposed to have identical release rates of ≈ 1 mg/d. However, we observed much higher responses to GA alone in 2004 than in 2003 (29.5 and 7.8 adults per trap, respectively), with significantly more plum curculios captured in traps baited with GA alone compared with unbaited traps in 2004 (Table 3), leading us to believe that 2004 dispensers may have had a higher release rate of GA, resulting in increased captures. Alternatively, an increased population density may account for higher captures in 2004, although captures in unbaited traps were similar in 2003 and 2004 (9.0 and 13.8 adults per trap, respectively). Another possibility may be related to pheromone chemistry and composition of lures deployed in 2003 and 2004. In both years, we deployed a racemic mixture of GA, (+)-(1R,2S)-1-methyl-2-(1-methylethenyl) cyclobutaneacetic acid (originally identified as the attractive component from male plum curculios), in combination with its (-)-enantiomer. Although a racemic mixture attracted plum curculios in original studies by Eller and Bartelt (1996), no published studies have reported how plum curculios respond to GA when the (-)-enantiomer is removed. In other species such as the almond bark beetle, *Scolytus amygdali* Geurin-Menneville, a racemic mixture of the four stereoisomers of the male pro-

duced aggregation pheromone, 4-methyl-3-heptanol, was less attractive than the single major component, (3S,4S)-4-methyl-3-heptanol (Ben-Yehuda et al. 2002), with two of these stereoisomers serving to inhibit male response to the single major component in combination with a synergist (Zada et al. 2004). Perhaps in our studies, differences in the percentage of formulated material consisting of the single major component of plum curculio aggregation pheromone, the (+)-GA optical isomer, resulted in different levels of attraction to GA lures in 2003 and 2004.

One of the problems with deployment of benzaldehyde in low-density polyethylene vials under field conditions is that it will solidify into crystals of *trans*-stilbene and benzoic acid presumably because of clogging of pores in the low-density polyethylene lamella. Compared with Ben(s) + GA, in which no provisions were taken to protect benzaldehyde from field degradation, Ben(TCB) + GA (formulated in UV-resistant vials with TCB) and Ben(nr) + GA (benzaldehyde alone in UV-resistant vials only) fared much better in 2003 with 60% and 38% higher captures, respectively. Furthermore, the addition of TCB allowed us to use the same lures throughout the season without replacement or any signs of degradation. In fact, we observed a significantly greater response to traps baited with Ben(TCB) + GA compared with unbaited traps in 2003 (RI = 35.1) and 2004 (RI = 35.8; Tables 1 and 2). In 2004, we observed similar responses among Ben(TCB) + GA, Ben(nr) + GA, and Ben(r) + GA, indicating that UV-resistant vials used for each of these treatments may be the most important formulation improvement, although we also observed less crystalline residue on Ben(TCB) vials than other Ben treatments.

Combined results point to new directions for research regarding host plant-based attractant for plum curculio. They include (1) on-tree collections of volatiles released by foliar, woody, and fruiting structures, particularly from plum, to create a more powerful bait; (2) release rate studies associated with multiple component blends; (3) a second look at the pheromone, perhaps refining a racemic mixture. More powerful baits used in conjunction with traps (Johnson et al. 2002, Prokopy et al. 2003, Leskey and Wright 2004a) or within the canopies of trees themselves, (i.e., using a trap-tree approach; Prokopy et al. 2003) could improve our ability to monitor plum curculio activity within commercial orchards and enable growers to assess the need for and timing of insecticide application.

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